DP Barcodes: 425262 and 425264 MRID No.: 49525001

DATA EVALUATION RECORD
HONEY BEE - ACUTE CONTACT and ORAL LC_{50}TEST
OECD 213 and 850.3020

1. **CHEMICAL:** Pyraclostrobin and Metconazole PC Code Nos.: 099100 and 125619

2. **TEST MATERIAL:** BAS 556 02 F Purity: 13.34% (pyraclostrobin)
5.03% (metconazole)

3. **CITATION**

Authors:	Franke M.
Title:	Acute Toxicity of BAS 556 02 F to the Honeybee *Apis mellifera* L. Under Laboratory Conditions
Study Completion Date:	December 1, 2014
Laboratory:	BioChem agrar Labor for biologische und chemische Analytik GmbH Kupferstrasse 6 04827 Gerichshain, Germany
Sponsor:	BASF SE, 67056 Ludwigshafen, Germany
Laboratory Report ID:	14 10 48 038 B
MRID No.:	49525001
DP Barcode:	425262 & 425264

4. **REVIEWED BY:** John Marton, Ph.D., Environmental Scientist, CDM Smith

Signature:  **Date:** 4/2/15

APPROVED BY: Teri S. Myers, Ph.D., Environmental Scientist, CDM Smith

Signature:  **Date:** 4/14/15

5. **APPROVED BY:** Meghan Radtke, Ph.D., Biologist, OPP/EFED/ERB-1

Signature:  **Date:** 4/21/15

6. **DISCLAIMER:** This document provides guidance for EPA and PMRA reviewers on how to complete a data evaluation record after reviewing a scientific study concerning the acute toxicity of a pesticide to honey bees via oral and contact exposure routes. It is not intended to prescribe conditions to any external party for conducting this study nor to establish absolute criteria regarding the assessment of whether the study is scientifically sound and whether the study satisfies any applicable data requirements. Reviewers are expected to review and to determine for each study, on a case-by-case basis, whether it is scientifically sound and provides sufficient

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conditions may be accepted, if appropriate; similarly, studies that meet all of the conditions may be rejected, if appropriate. In sum, the reviewer is to take into account the totality of factors related to the test methodology and results in determining the acceptability of the study.

7. STUDY PARAMETERS:

Scientific Name of Test Organism:	*Apis mellifera* L.
Age of Test Organism at Test Initiation:	Young adult worker bees (3-5 weeks)
Type of Concentrations:	Nominal (contact), actual intake (oral)
Definitive Test Duration:	48 hours for each test

8. CONCLUSIONS:

The honey bee, *Apis mellifera* L., was exposed to BAS 556 02 F (pyraclostrobin [13.34%] and metconazole [5.03%]) for 48 hours in the oral and the contact test. The nominal contact concentrations were 33.3, 66.6, 133.1, 266.2, and 532.5 µg formulation/bee, corresponding to 1.67, 3.35, 6.69, 13.4, and 26.8 µg metconazole/bee, and 4.44, 8.88, 17.8, 35.5, and 71.0 µg pyraclostrobin/bee. Nominal concentrations in the oral test were 16.6, 33.3, 66.6, 133.1, and 266.2 µg formulation/bee, corresponding to 0.835, 1.67, 3.35, 6.69, and 13.4 µg metconazole/bee, and 2.21, 4.44, 8.88, 17.8, and 35.5 µg pyraclostrobin/bee. The actual intake concentrations were identical to nominal. By 48 hours in the oral test, mortality was 0% in the control and 0, 3.3, 40.0, 86.7, and 100% in the 16.6, 33.3, 66.6, 133.1, and 266.2 µg formulation/bee. Impaired movement was observed among bees in the 66.6-266.2 µg formulation/bee treatment groups after 4 hours of exposure; all surviving bees appeared normal and healthy at all other observations. By 48 hours in the contact test, mortality was 3.3% in both the negative and Tween controls, and 0, 0, 0, 13.3, and 23.3% in the 33.3, 66.6, 133.1, 266.2, and 532.5 µg formulation/bee treatment groups, respectively. After 4 hours of exposure, impaired mobility was observed in the 133.1 to 532.5 µg formulation/bee treatment groups; all surviving bees appeared normal and healthy at all other observations.

Results - Oral Test:

LD_{50} = 76.6 µg formulation/bee	95% C.I.: 65.4-89.7 µg formulation/bee
NOAEC = 33.3 µg formulation/bee	
LD_{50} = 3.85 µg metconazole/bee	95% C.I.: 3.29-4.51 µg metconazole/bee
LD_{50} = 10.2 µg pyraclostrobin/bee	95% C.I.: 8.72-12.0 µg pyraclostrobin/bee
Probit Slope = 4.97	95% C.I.: 3.50-6.43

Results - Contact Test:

LD_{50} > 532.5 µg formulation/bee	95% C.I.: N/A
NOAEC = 133.1 µg formulation/bee	

LD_{50} > 26.8 µg metconazole/bee 95% C.I.: N/A
LD_{50} > 71.0 µg pyraclostrobin/bee 95% C.I.: N/A
Probit Slope: N/A 95% C.I.: N/A

As a result, the formulated product, BAS 556 02 F, and the two active ingredients, metconazole and pyraclostrobin, are categorized as "practically non-toxic" to honey bees *on an acute contact basis* and "moderately toxic" *on an acute oral basis*.

This study is scientifically sound and is classified as "acceptable."

9. ADEQUACY OF THE STUDY:

A. Classification: Acceptable

B. Rationale: N/A

C. Repairability: N/A

10. GUIDELINE DEVIATIONS: This study was conducted following guidelines outlined in OECD 213 and 850.3020. No deviations were noted.

11. SUBMISSION PURPOSE: This study was submitted to provide data on the effects of BAS 556 02 F to honey bees (*Apis mellifera* L.) following acute oral and contact exposure for the purpose of updating the pollinator language on the label.

12. MATERIALS AND METHODS:

A. Test Organisms

Guideline Criteria	Reported Information
Species: Species of concern (*Apis mellifera, Megachile rotundata,* or *Nomia melanderi*)	*Apis mellifera* L.
Age at beginning of test:	Young adult worker bees (3-5 weeks)
Supplier:	Bienenfarm Kern GmbH Rehbacher Anger 10, 04249 Leipzig, Germany.
All bees from the same source?	Yes

B. Test System

Guideline Criteria	Reported Information
Cage size adequate?	Yes. Disposable cardboard cages with holes in the bottom for ventilation and a glass plate in front. 95 x 50 x 65 mm (length x width x height)
Lighting:	Constant darkness throughout the test (diffuse artificial light of ~100 lx during handling and assessments)
Temperature:	24.8-25.2°C
Relative humidity:	58-61%

C. Test Design

Guideline Criteria	Reported Information
Range finding test?	The study author reported that oral and contact range-finding studies were performed. However, no details were provided.
Reference toxicant test?	Dimethoate: 0.086, 0.123, 0.175, and 0.250 µg ai/bee
Method of administration:	Oral test: 50% w/v sucrose solution via feeding tube Contact test: 2 µL droplet applied to the dorsal thorax following anaesthetization with CO_2. The wetting agent Tween® 80 (1.0% v/v) was included in the application.

Guideline Criteria	Reported Information
Nominal doses:	**Oral test:** Formulation: 16.6, 33.3, 66.6, 133.1, and 266.2 µg/bee Metconazole: 0.835, 1.67, 3.35, 6.69, and 13.4 µg ai/bee Pyraclostrobin: 2.21, 4.44, 8.88, 17.8, and 35.5 µg ai/bee **Contact test:** Formulation: 33.3, 66.6, 133.1, 266.2, and 532.5 µg/bee Metconazole: 1.67, 3.35, 6.69, 13.4, and 26.8 µg ai/bee Pyraclostrobin: 4.44, 8.88, 17.8, 35.5, and 71.0 µg ai/bee
Controls: Negative control and/or diluent/solvent control	Oral test: 50% w/v sucrose solution Contact test: deionized water (negative control) and deionized water with wetting agent (1.0% (v/v) Tween80®) (vehicle/carrier control)
Number of replicates per treatment group:	Oral test: 3 (10 bees per rep) Contact test: 3 (10 bees per rep)
Solvent: The following solvents: acetone, dimethylformamide, triethylene glycol, methanol, ethanol.	Oral test: N/A Contact test: N/A

Guideline Criteria	Reported Information
Feeding:	Oral test: appropriately treated 50% (w/v) sucrose solution for 1.5 hours, then untreated sucrose solution thereafter until test termination Contact test: 50% (w/v) sucrose solution Test solutions were provided via feeding tubes (glass ampoule) inserted through a hole in the test chambers.
Observations period:	Oral test: 4, 24, and 48 hours Contact test: 4, 24, and 48 hours

13. REPORTED RESULTS:

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	Yes. Signed and dated No Data Confidentiality, GLP, and Quality Assurance statements were provided. This study was conducted in compliance with the Principles of Good Laboratory Practice, Annex 1 to Chemicals Act of Federal Republic of Germany in the current version based on the "OECD Principles of Good Laboratory Practice" (as revised in 1997; Environment Directorate, Organization for Economic Cooperation and Development, Paris 1998) and the Directive 2004/10EC of 11 February 2004 amending Council Directive 87/18/EEC, which are accepted by regulatory authorities throughout the European Union, the United States of America (FDA and EPA) and Japan (MHLW, MAFF, and METI).

Guideline Criteria	Reported Information
Control performance:	Oral test: 0% mortality Contact test: 3.3% mortality in the negative and Tween controls
Raw data included:	Adequate data were provided to verify statistical analysis.
Signs of toxicity (if any) were described?	Yes

Mortality - Oral Test

Dosage µg formulation/bee (actual intake)	**No. of Bees**	**Percent Mortality (%)**	
		Hour of Study	
		24	**48**
Test Substance			
Negative Control	30	0	0
16.6	30	0	0
33.3	30	0	3.3
66.6	30	36.7	40.0
133.1	30	86.7	86.7
266.2	30	100	100
Toxic Standard			
0.086	30	3.3	3.3
0.123	30	16.7	36.7
0.175	30	66.7	73.3
0.250	30	100	100

Observations: No mortalities occurred in the control or 16.6 µg formulation/bee treatment groups throughout the definitive exposure period. Mortality in the remaining treatment groups exhibited a dose-dependent pattern, and the study author reported a 48-hour LD_{50} value of 76.6 (65.8-89.0) µg formulation/bee. The 48-hr LD_{50} of the dimethoate reference item was reported to be 0.140 (0.128-0.152) µg ai/bee.

Sub-lethal effects were only noted at the 4-hour observation. Impaired locomotion was observed in 15, 7, and 4 bees in the 66.6, 133.1, and 266.2 µg formulation/bee treatment groups, respectively. After 24 and 48 hours of exposure, all surviving honey bees appeared normal and healthy.

Mortality - Contact Test

Dosage µg formulation/bee (actual contact)	**No. of Bees**	**Percent Mortality (%)**	
		Hour of Study	
		24	**48**
Test Substance			
Negative Control	30	0	3.3
Tween Control	30	0	3.3
33.3	30	0	0
66.6	30	0	0
133.1	30	0	0
266.2	30	10.0	13.3
532.5	30	23.3	23.3
Toxic Standard			
0.086	30	0	3.3
0.123	30	3.3	6.7
0.175	30	30.0	46.7
0.250	30	90.0	90.0

Observations: A single mortality was observed in the negative and Tween controls after 48 hours of exposure. No mortalities were observed in the 33.3-133.1 µg formulation/bee treatment groups, and mortality was 13.3 and 23.3% in the 266.2 and 532.5 µg formulation/bee treatment groups, respectively. Since mortality did not exceed 50%, the study author reported an LD_{50} value of >532.5 µg formulation/bee. The LD_{50} for the dimethoate reference was 0.183 (0.170-0.198) µg ai/bee.

Similar to the oral exposure, sub-lethal effects were restricted to the 4-hour observation, during which impaired locomotion was observed in 1, 6, and 11 honey bees in the 133.1, 266.2, and 532.5 µg formulation/bee treatment groups, respectively. No other sub-lethal effects were observed in the controls or treatment groups throughout the 48-hour exposure.

Statistical method: When applicable, mortality for each concentration was corrected according to Abbott (1925) modified by Schneider Orelli (1947). Mortality was assessed using Fisher's Exact Binomial test (with Bonferroni Correction). The LD_{50} and 95% confidence limits were calculated with the probit analysis, using linear maximum likelihood regression for calculation of the test item and reference item. The statistical calculations were performed with ToxRat Profession 3.0 beta.

Reported Statistical Results - Oral Test:

LC_{50}: 76.6 µg formulation/bee	95% C.I.: 65.8-89.0 µg formulation/bee
Probit Slope: 4.97±0.56	95% C.I.: Not Reported

Reported Statistical Results - Contact Test:

LD_{50}: >532.5 µg formulation/bee	95% C.I.: N/A
Probit Slope: N/A	95% C.I.: N/A

14. VERIFICATION OF STATISTICAL RESULTS:

Statistical method: The 48-hr LC_{50} value for the oral test was estimated using the probit analysis via CETIS statistical software version 1.8.7.12 with database backend settings implemented by EFED on 3/25/14. The reviewer used the actual uptake concentrations based on the formulated product and then converted the toxicity values based on the individual purities of pyraclostrobin and metconazole. Mortality did not exceed 50% in the contact test, therefore, the contact LD_{50} was visually determined to be greater than the highest treatment level.

Results - Oral Test:

LD_{50} = 76.6 µg formulation/bee	95% C.I.: 65.4-89.7 µg formulation/bee

LD_{50} = 3.85 µg metconazole/bee	95% C.I.: 3.29-4.51 µg metconazole/bee
LD_{50} = 10.2 µg pyraclostrobin/bee	95% C.I.: 8.72-12.0 µg pyraclostrobin/bee
Probit Slope = 4.97	95% C.I.: 3.50-6.43

Results - Contact Test:

LD_{50} > 532.5 µg formulation/bee	95% C.I.: N/A
LD_{50} > 26.8 µg metcoanzole/bee	95% C.I.: N/A
LD_{50} > 71.0 µg pyraclostrobin/bee	95% C.I.: N/A
Probit Slope: N/A	95% C.I.: N/A

15. REVIEWER'S COMMENTS:

The reviewer's results based on the formulated product were comparable to those reported by the study author. However, the reviewer also corrected these values for the individual active ingredients, pyraclostrobin and metconazole, based on their respective purities. Therefore, the reviewer's results are reported in the Conclusions section of this DER.

The in-life portion of the definitive toxicity test was conducted from October 1 to 3, 2014.

16. REFERENCES:

Abbott WS. 1925. A method of computing the effectiveness of an insecticide. J. Econ. Entomol. 18, 265-267.

Finney DJ. 1971. Probit Analysis, 3rd Edition. London: Cambridge University Press.

Ratte M. 2010. ToxRat Professional 3.0 beta. ToxRat Solutions GmbH, Naheweg 15, 52477 Alsdorf, Germany.

Schneider-Orelli O. 1947. Entomologisches Praktikum. H.R. Sauerlander. Aarau, Switzerland.

OCSPP 850.3020 Acute Honey Bee Test **BioChem Agrar**

Batch ID:	16-8726-1164	**Test Type:**	Mortality (48-h)	**Analyst:**	
Start Date:	01 Oct-14	**Protocol:**	OCSPP 850.3020 Acute Honey Bee	**Diluent:**	Aqueous Sucrose
Ending Date:		**Species:**	Apis mellifera	**Brine:**	Not Applicable
Duration:	NA	**Source:**	Bienenfarm Kern	**Age:**	Adlt

Sample ID:	18-5011-1046	**Code:**	49525001 oral	**Client:**	CDM Smith
Sample Date:	01 Oct-14	**Material:**	BAS 556 02 F	**Project:**	Fungicide
Receive Date:		**Source:**	BASF Corporation		
Sample Age:	NA	**Station:**			

Batch Note: PC Code 099100 (pyraclostrobin, 13.34%) & 125619 (metconazole, 5.03%)
Sample Note: PC Code 099100 (pyraclostrobin, 13.34%) & 125619 (metconazole, 5.03%)

Point Estimate Summary

Analysis ID	Endpoint	Level	µg/bee	95% LCL	95% UCL	TU	Method
03-4742-5873	48hSurvival	EC5	35.7	24.8	44.5		Linear Regression (MLE)
		EC10	42.3	31.1	51.2		
		EC15	47.4	36.2	56.5		
		EC20	51.8	40.8	61.1		
		EC25	56	45.1	65.6		
		EC40	68.1	57.2	79.2		
		EC50	76.6	65.4	89.7		
17-8841-7396	48hSurvival	EC50	76.5	65.3	89.5		Spearman-Kärber

48hSurvival Summary

C-µg/bee	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Negative Control	3	1	1	1	1	1	0	0	0.0%	0.0%
16.6		3	1	1	1	1	1	0	0	0.0%	0.0%
33.3		3	0.967	0.823	1	0.9	1	0.0333	0.0577	5.97%	3.33%
66.6		3	0.6	0.6	0.6	0.6	0.6	0	0	0.0%	40.0%
133.1		3	0.133	0	0.277	0.1	0.2	0.0333	0.0577	43.3%	86.7%
266.2		3	0	0	0	0	0	0	0		100.0%

48hSurvival Detail

C-µg/bee	Control Type	Rep 1	Rep 2	Rep 3
0	Negative Control	1	1	1
16.6		1	1	1
33.3		1	0.9	1
66.6		0.6	0.6	0.6
133.1		0.2	0.1	0.1
266.2		0	0	0

OCSPP 850.3020 Acute Honey Bee Test **BioChem Agrar**

Analysis ID:	03-4742-5873	**Endpoint:**	48hSurvival	**CETIS Version:**	CETISv1.8.7
Analyzed:	03 Apr-15 12:16	**Analysis:**	Linear Regression (MLE)	**Official Results:**	Yes
Batch ID:	16-8726-1164	**Test Type:**	Mortality (48-h)	**Analyst:**	
Start Date:	01 Oct-14	**Protocol:**	OCSPP 850.3020 Acute Honey Bee	**Diluent:**	Aqueous Sucrose
Ending Date:		**Species:**	Apis mellifera	**Brine:**	Not Applicable
Duration:	NA	**Source:**	Bienenfarm Kern	**Age:**	Adlt

Linear Regression Options

Model Function	Threshold Option	Threshold	Optimized	Pooled	Het Corr	Weighted
Log-Normal [NED=A+B*log(X)]	Control Threshold	1E-07	No	No	No	Yes

Regression Summary

Iters	LL	AICc	BIC	Mu	Sigma	Adj R2	F Stat	Critical	P-Value	Decision(α:5%)
22	-36.5	77.9	78.9	1.88	0.201	0.977	0.185	3.26	0.9420	Non-Significant Lack of Fit

Point Estimates

Level	µg/bee	95% LCL	95% UCL
EC5	35.7	24.8	44.5
EC10	42.3	31.1	51.2
EC15	47.4	36.2	56.5
EC20	51.8	40.8	61.1
EC25	56	45.1	65.6
EC40	68.1	57.2	79.2
EC50	76.6	65.4	89.7

Regression Parameters

Parameter	Estimate	Std Error	95% LCL	95% UCL	t Stat	P-Value	Decision(α:5%)
Slope	4.97	0.747	3.5	6.43	6.64	<0.0001	Significant Parameter
Intercept	-9.36	1.42	-12.1	-6.58	-6.6	<0.0001	Significant Parameter

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Model	128.5424	128.5424	1	732	<0.0001	Significant
Lack of Fit	0.163040	0.040760	4	0.185	0.9418	Non-Significant
Pure Error	2.645889	0.220491	12			
Residual	2.808929	0.175558	16			

Residual Analysis

Attribute	Method	Test Stat	Critical	P-Value	Decision(α:5%)
Goodness-of-Fit	Pearson Chi-Sq GOF	2.81	26.3	0.9999	Non-Significant Heterogenity
	Likelihood Ratio GOF	3.19	26.3	0.9997	Non-Significant Heterogenity
Variances	Mod Levene Equality of Variance	0.826	4.39	0.5738	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.834	0.897	0.0047	Non-normal Distribution
	Anderson-Darling A2 Normality	1.62	2.49	<0.0001	Non-normal Distribution

48hSurvival Summary

			Calculated Variate(A/B)								
C-µg/bee	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect	A	B
0	Negative Control	3	1	1	1	0	0	0.0%	0.0%	30	30
16.6		3	1	1	1	0	0	0.0%	0.0%	30	30
33.3		3	0.967	0.9	1	0.0333	0.0577	5.97%	3.33%	29	30
66.6		3	0.6	0.6	0.6	0	0	0.0%	40.0%	18	30
133.1		3	0.133	0.1	0.2	0.0333	0.0577	43.3%	86.7%	4	30
266.2		3	0	0	0	0	0		100.0%	0	30

OCSPP 850.3020 Acute Honey Bee Test **BioChem Agrar**

Analysis ID:	03-4742-5873	**Endpoint:**	48hSurvival	**CETIS Version:**	CETISv1.8.7
Analyzed:	03 Apr-15 12:16	**Analysis:**	Linear Regression (MLE)	**Official Results:**	Yes

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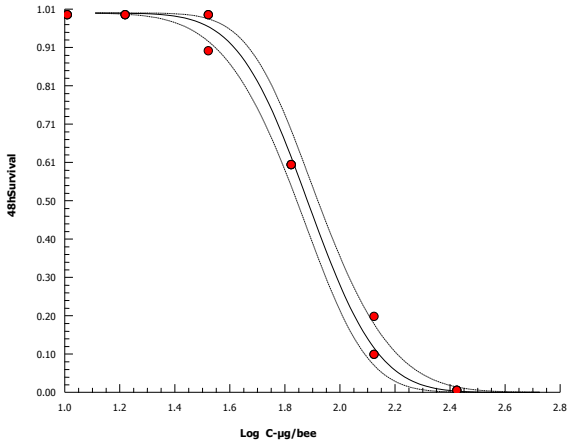

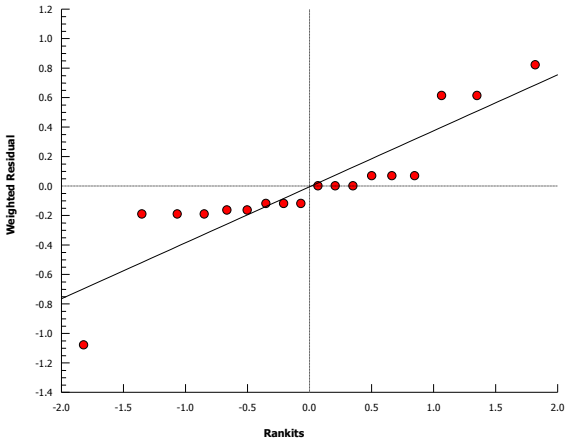

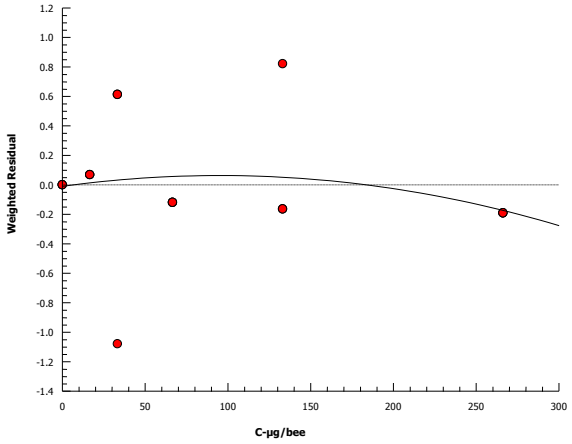

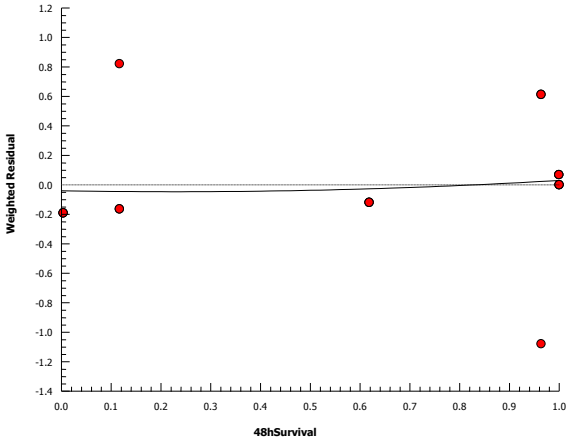

OCSPP 850.3020 Acute Honey Bee Test **BioChem Agrar**

Analysis ID:	17-8841-7396	**Endpoint:**	48hSurvival	**CETIS Version:**	CETISv1.8.7
Analyzed:	03 Apr-15 12:16	**Analysis:**	Untrimmed Spearman-Kärber	**Official Results:**	Yes
Batch ID:	16-8726-1164	**Test Type:**	Mortality (48-h)	**Analyst:**	
Start Date:	01 Oct-14	**Protocol:**	OCSPP 850.3020 Acute Honey Bee	**Diluent:**	Aqueous Sucrose
Ending Date:		**Species:**	Apis mellifera	**Brine:**	Not Applicable
Duration:	NA	**Source:**	Bienenfarm Kern	**Age:**	Adlt

Spearman-Kärber Estimates

Threshold Option	Threshold	Trim	Mu	Sigma	EC50	95% LCL	95% UCL
Control Threshold	0	0.00%	1.88	0.0342	76.5	65.3	89.5

48hSurvival Summary **Calculated Variate(A/B)**

C-µg/bee	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect	A	B
0	Negative Control	3	1	1	1	0	0	0.0%	0.0%	30	30
16.6		3	1	1	1	0	0	0.0%	0.0%	30	30
33.3		3	0.967	0.9	1	0.0333	0.0577	5.97%	3.33%	29	30
66.6		3	0.6	0.6	0.6	0	0	0.0%	40.0%	18	30
133.1		3	0.133	0.1	0.2	0.0333	0.0577	43.3%	86.7%	4	30
266.2		3	0	0	0	0	0		100.0%	0	30

Graphics